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SOLUBILIZATION OF ADENOSINE TRIPHOSPHATASE ASSOCIATED WITH HERPES SIMPLEX VIRUS

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Summary. — The nonionic detergent Triton X-100 was used for the solubilization of Mg^{2+}-dependent adenosine triphosphatase (Mg^{2+}ATPase) associated with mature herpes simplex virus (HSV) particles purified from infected rabbit lung (ZP) cells. The solubilization was the best at pH 8.1 with a Triton X-100 to protein ratio of 10. The solubilized enzyme splited ATP at the greatest rate at pH from 7.9 to 8.6. pH $>$ 8.6 during extraction had a deleterious effect on the enzyme. In the presence of NaCl significantly more proteins were extracted but the enzyme was slightly inhibited. No enhancement of the enzyme activity after detergent treatment and the relatively mild conditions for extraction indicated that the enzyme is not too firmly associated with the surface of the herpesvirions.

Key words: herpes simplex virus; virions; adenosine triphosphatase

Introduction

Previously (Matis *et al.*, 1975) we showed that Mg^{2+}-dependent adenosine triphosphatase (Mg^{2+} ATPase) remained associated with purified enveloped particles of herpes simplex virus (HSV) isolated from infected rabbit lung (ZP) cells. The association of Mg^{2+} ATPase with the viral envelope was inferred from the fact that the purified unenveloped viral particles (nucleocapsids) were devoid of this enzyme activity. The finding that the ATPase associated with the viral envelope and nuclear membranes of host cells share mary common properties (Matis *et al.*, 1978) supports the well-established participation of these membranes in the envelopment of herpesviruses (Siegert and Falke, 1966; Darlington and Moss, 1968; Nii *et al.*, 1968).

In the present work we studied the solubilization of this enzyme by the nonionic detergent Triton X-100.

Materials and Methods

Purification of virus. Virions of the HSZP strain of HSV (Szántó, 1960) were purified from infected rabbit lung (ZP) cells as described (Matis *et al.*, 1975). Briefly, the clarified virus suspension was subjected to two cycles of high (60000×g, 50 min) and low (2000×g, 25 min) speed centrifugation. Then followed centrifugation through a discontinuous Ficoll gradient prepared

Table 1. Effect of Triton X-100 concentration on solubilization of ATPase

Triton X-100 concentration (%)	Triton X-100 to protein ratio	Protein in supernatant (μg)	Activity in supernatant (μg P_i/hr)	Specific activity* (μg P_i/hr/mg protein
0.010	1	4	0	0
0.025	2.5	9	0	0
0.050	5.0	10	1.1	110
0.075	7.5	21	3.7	175
0.100	10.0	23	4.3	187
0.150	15.0	29	4.2	145
0.200	20.0	28	2.1	75

* Specific activity of virion proteins before Triton X-100 treatment was 40 μg P_i/hr/mg protein.

To purified virions resuspended in water (protein content 100 μg), equal volumes of solutions containing various amounts of Triton X-100 in 0.02 M Tris-HCl were added. The mixtures were kept at 4 °C for 60 min and then centrifuged at 100000 × g for 60 min. Thereafter 200 μl of the supernatants were assayed for ATPase activity. The sediments were washed with phosphate buffered saline (PBS), pelleted and resuspended in 1 ml of PBS. Portions of 500 μl were taken for protein determination. The protein amounts present in supernatants were calculated as follows: total proteins (100 μg) minus proteins determined in sediments.

by overlaying equal volumes of 40, 30, 20, and 10% (w/w) Ficoll solutions in water (68000 × g, 45 min). Viral particles at the interfaces between 10—20 and 20—30% Ficoll solutions were collected, freed of Ficoll, layered on a 5—55% (w/w) sucrose-D_2O density gradient and centrifuged (140000 × g, 6 hr). The band of virions below the middle of the tube was collected, diluted with water and pelleted. The virions were resuspended in water and stored at 4 °C.

Reagents. Disodium salt of adenosine-5'-triphosphate (ATP) was purchased from Calbiochem (U.S.A.). It was converted to Tris-salt by passing through a column of Dowex 50W × 12 (200/400 mesh in H^+ form) and subsequent neutralization. The content of ATP in the Tris-salt thus prepared was at least 98% as determined by the Calbiochem ATP STAT-PACK. Triton X-100 was from BDH (England). All other reagents used were of analytical grade and purchased from Lachema, Czechoslovakia.

Protein content of the virus suspensions and sediments after high speed centrifugation was determined according to Lowry *et al.*, using bovine serum albumine as a standard.

Solubilization of ATPase. The standard technique was as follows: to purified virions resuspended in distilled water, an equal volume of Triton X-100 solution in 20 mM Tris-HCl pH 8.1 was added (total volume 1 ml, the ratio of Triton X-100 to protein was 10). The suspension was kept at 4 °C

Table 2. Effect of NaCl on the extractability of virion proteins

M NaCl in incubation mixture	Protein in supernatant (μg)	P_i in supernatant (μg)	Specific activity (μg P_i/hr/mg protein)
0	18	4.1	228
0.1	17	3.8	224
0.3	38	4.0	105
0.5	37	3.6	97
1.0	39	3.8	97

Solubilization and estimation of P_i as described in Materials and Methods.

for 60 min and then centrifuged at 100000×g for 60 min. The supernatant was assayed for ATPase activity. Details will be given along with the results.

Determination of adenosine triphosphatase (ATPase) activity. The standard incubation mixture for the determination of ATPase activity contained in a final volume of 1 ml: 40 μmoles Tris-HCl, pH 8.1, 1 μmole $MgCl_2$ 1 μmole Tris-ATP, and Triton X-100-solubilized material. Incubation lasted 60 min at 37 °C. The enzymatic activity was estimated by determining the amount of inorganic phosphate (P_i) liberated as described by Mozersky et al. (1966) and modified by Matis et al. (1975). Triton X-100 in the incubation mixtures did not interfere with the P_i determination.

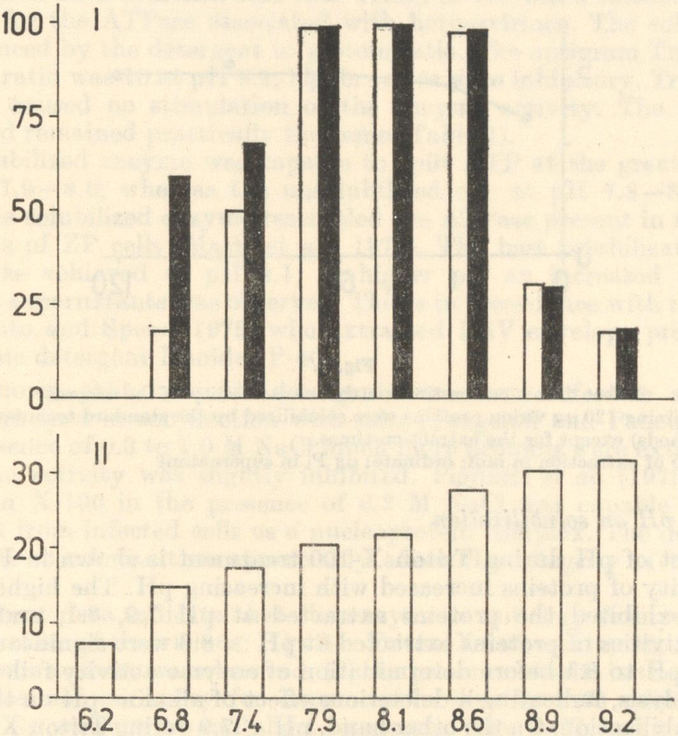

Fig. 1.

ATPase activities of virion proteins extracted at various pH

Samples (1 ml) containing 100 μg virion proteins, 0.1% Triton X-100, 10 mM Tris-HCl buffer at various pH (at pH < 7.9, 10 mM Tris-maleate buffer) were incubated for 60 min at 4 °C and centrifuged (100000×g, 60 min). Supernatants were assayed for activity (I) and protein content (II) — (for details see text to Table 1).

Abscissa: pH values during extraction.

Ordinate I: Specific activities (μg P_i/hr/mg protein) in % of that of virion proteins extracted and assayed at pH 8,1, which was taken for 100%. Empty columns: activities assayed at pH used during extraction. Shaded columns: virion proteins extracted at given pH, but assayed for activity at pH 8.1.

Ordinate II: μg virion proteins in the supernatant.

Results

Typical results of one of the experiments on the effect of Triton X-100 concentration on ATPase solubilization are summarized in Table 1. The activity and the amount of proteins in the supernatant increased with increasing Triton X-100 concentration. The specific activity was the highest at a Triton X-100 to protein ratio of 10. At the ratio of 20, no further solubilization of proteins was observed and the activity was lowered to 50%.

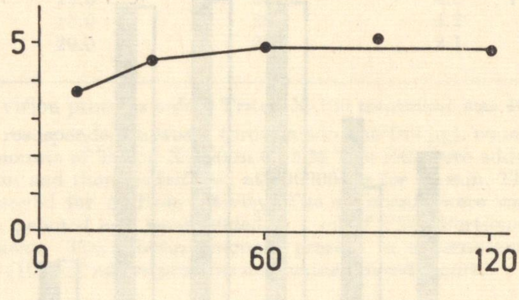

Fig. 2.

Effect of incubation time on the extractability of the enzyme
Samples containing 120 μg virion proteins were solubilized by the standard technique (see Materials and Methods) except for the extraction times.
Abscissa: time of extraction in min; ordinate: μg P_i in supernatant

Effect of pH on solubilization

The effect of pH during Triton X-100 treatment is shown in Fig. 1. The extractability of proteins increased with increasing pH. The highest specific activities exhibited the proteins extracted at pH 7.9, 8.1, and 8.6. The specific activities of proteins extracted at pH > 8.6 were significantly lower. A shift in pH to 8.1 before determination of enzyme activity failed to affect ATP hydrolysis, indicating a deleterious effect of alkaline pH on the enzyme during solubilization. On the other hand, pH < 7.9 during Triton X-100 treatment had practically no influence on the enzymatic activity, but the ability to extract the enzyme was lowered.

Effect of NaCl on enzyme extraction

Incubation mixtures with various concentrations of NaCl were tested. Approximately twofold amounts of proteins were extracted at 0.3—1.0 M NaCl concentrations but the ability of the extracted proteins to hydrolyze ATP was slightly decreased (Table 2).

Time-dependence of the enzyme extraction. The extraction of the enzyme was a relatively rapid process (Fig. 2). After 10 min the bulk of the enzymatic activity was found in the supernatant. Under the experimental conditions used, no further extraction was observed after 60 min of incubation.

Discussion

The supernatant fraction of the detergent-treated enzyme obtained following centrifugation at 100000xg for 60 min has been designated as soluble enzyme. In preliminary experiments, however, the enzyme did not sediment upon a prolonged centrifugation (5 hr). The amount of proteins in the supernatants was not estimated directly because the detergent interfered in the determination of proteins by Lowry's procedure.

The present data demonstrated that Triton X-100 was a suitable solubilizing agent for the ATPase associated with herpesvirions. The solubilization was influenced by the detergent to protein ratio. The optimum Triton X-100 to protein ratio was 10 at pH 8.1, higher ratios were inhibitory. Triton X-100 treatment caused no stimulation of the enzyme activity. The amount of P_i liberated remained practically the same (Table 1).

The solubilized enzyme was capable to split ATP at the greatest rate at pH from 7.9–8.6, whereas the unsolubilized one at pH 7.8–8.0. In this respect, the solubilized enzyme resembled the ATPase present in the nuclear membranes of ZP cells (Matis et al., 1978). The best solubilization of the enzyme was achieved at pH 8.1; at higher pH an increased amount of proteins in supernatants was observed. This is in accordance with the findings of Sarmiento and Spear (1979) who extracted HSV envelope proteins with the nonionic detergent Nonidet P-40.

It is known that nonionic detergents are more effective solubilizing agents when used in conjunction with salts (Tzagoloff and Penefsky, 1971). In the presence of 0.3 to 1.0 M NaCl much more proteins were extracted, but the enzyme activity was slightly inhibited. Pignatti *et al.* (1979) reported that Triton X-100 in the presence of 0.2 M NaCl was capable to extract HSV DNA from infected cells as a nucleoprotein complex. The detergent in the presence of salt can thus appreciably affect the integrity of the herpesvirions.

The present data indicate that the enzyme is not very firmly associated with the surface of the virions: (1) after Triton X-100 treatment, no increase of the enzyme activity was observed; (2) the presence of salts in the extraction solution was not necessary for enzyme solubilization; (3) relatively mild conditions for extraction and short exposures were sufficient to solubilize the bulk of the enzyme and (4) the extraction of proteins was not quantitative (acrylamide gel electrophoresis studies are in progress). In spite of this, the insoluble material in the pellets did not exhibit enzyme activity.

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